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Identification of Microbial Contamination of Popular Fruits of Bangladesh and Assessment the Effects of Alternative Preservatives Instead of Formalin

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Abstract The demand of fresh fruits is increasing as consumers are striving to eat healthy diets. Most of the fruits are generally eaten without further processing. During growth, harvest, transportation and handling, fruits become contaminated with pathogens from human or animal source. The study was aimed to isolate, identify the fruit surface microorganisms and to determine the microbial growth inhibitory effects of formalin, vinegar and salt. The fruits were washed with distilled water, vinegar, formalin and salt solution. These effluents were used as the sources of microbes. Results indicated the following bacterial growth pattern: *Klebsiella pneumoniae* (25%), *Escherichia coli* (21%), *Serratia marcescens* (12.5 %), *Pseudomonas aeruginosa* (17%), *Bacillus cereus* (16.5%) and *Staphylococcus aureus* (8%). Most of these isolated microorganisms are pathogenic to human. Due to increasing complications and health hazards for chemically synthesized preservative, consumers expect to get wholesome, fresh-like, and safe foods without addition of toxic preservatives (like formalin). A weak acid named acetic acid (vinegar) can be used to effectively reduce pathogenic and spoilage microorganisms present on fruit surfaces. From the study it might be concluded that the use of chemical decontaminants like vinegar (acetic acid) or salt might be an effective way to reduce the microbial load on fruit surfaces.

Keywords: *fruits, formalin, escherichia coli*

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1. Introduction

Fruits are being considered as nature's best gift for human beings. From the ancient period of time, people eat fruits to meet their hunger [3]. They are sweet or sour and many of them are edible in raw state, such as, mangoes, bananas, litchis, dates, rambai, guavas etc. Fruits are generally high in fiber, water, vitamins (A and C), sugars, minerals like Potassium (K) etc [8]. Fruits also contain various photochemical, which are required for proper long-term cellular health and disease prevention. Regular consumption of fruit is associated with reduced risks of cancer, cardiovascular disease (especially coronary heart disease), stroke, Alzheimer disease, cataracts, and some of the functional declines associated with aging [11]. Diets that include a sufficient amount of potassium from fruits and vegetables also help reduce the chance of developing kidney stones and may help reduce the effects of bone-loss. Fruits are also low in calories which would help lower one's calorie intake as part of a weight-loss diet [22]. Traditionally, fruits have been regarded as microbiologically safer than other unprocessed food items. But truly fresh fruits can carry an abundance of microorganisms on their surfaces. Not all microorganisms present on fruit surfaces can cause diseases but some microbes on fruits have been linked with illness. So the consumption of raw fruits may represent an important means by which new lineages of pathogenic bacteria are introduced into the human gastrointestinal system [5]. Pathogens that are frequently associated with fresh fruit originate, for the most part, from enteric environments - that is, they are found in the intestinal tract and fecal material of humans or animals. [10]. Most pathogens do not cause fruits to spoil, even at relatively high populations. In the absence of spoilage, high populations of pathogens may be achieved and the item may be consumed because it is not perceived as spoiled. In addition to directly causing disease, some microbes found on fruit surface may have other, less direct, impacts on human health. A vigorous population of

nonpathogenic microorganisms is potentially another barrier to reduce the number of microorganisms from fruit surface [14]. These microorganisms do not necessarily prevent the growth of pathogens but they do provide indicators of temperature abuse and age of the produce by causing detectable spoilage. Fruits can become contaminated with microbial pathogens by a wide variety of mechanisms. Contamination has occurred during production, harvest, processing, and transporting, as well as in retail and fruit service establishments and in the home kitchen [12]. Washing fruits thoroughly is a critical control point in reducing or eliminating contamination with microorganisms [1]. The fruit surface has long been considered a suitable environment for the growth of microorganisms. More generally, fruits surface microbes have impact on the rates of food spoilage. Preservatives are natural or synthetic substances that are added to products to retard spoilage due to whether, microbial growth or undesirable chemical changes [19]. They passively diffuse through the bacteria cell wall and internalizing into neutral pH dissociating into anions and protons. Release of the protons causes the internal pH to decrease which exert inhibitory effects on the bacteria [18]. Salt (sodium chloride) has been used to preserve fruits for thousands of years. Sodium chloride (NaCl) inhibits microbial growth by increasing osmotic pressure as well as decreasing the water activity in the micro-environment. The reduction of water activity due to the addition of salt and the presence of ions exerting osmotic pressure effect the growth of pathogenic or spoilage organisms. Most food borne bacteria, including *Clostridium botulinum*, *E. coli*, *Salmonella* spp., and the spoilage bacteria *Pseudomonas* spp., cannot grow below a water activity of 0.92. However, there are some species that tolerate lower minimum water activities: *Staphylococcus aureus* (0.83), and some spoilage yeasts (0.62). Molds (*Aspergillus* and *Penicillium*) tolerate lower water activities than most bacteria, ranging from 0.80 to 0.83 [23]. Hypo means under or less. A hypotonic solution is a medium whose concentration of solute (salt) is lower than that inside cell. For salt water solution anything below 0.9% is hypotonic. If fresh fruits are washed with hypotonic solution of salt as 0.5% NaCl, microorganism cell encounters a hypotonic environment, water will diffuse into the cell and the cell will begin to swell. Most of the microbial cells burst in hypotonic solution due to difference in osmotic pressure inside and outside the cells. The word 'Hyper' means above or more. A hypertonic solution is a medium having a higher concentration of solutes than inside the cell. Most microbial cells placed in a hypertonic solution shrink and collapse because water leaves the cells by osmosis [7]. The objectives of this study were to isolate the microorganisms from the surfaces of various types of popular fruits of Bangladesh and to investigate the inhibitory effects of formalin, vinegar (acetic acid) and salt (NaCl) on these isolated microbes.

2. Materials and Methods

2.1. Sample Collection

Six different kinds of fresh fruits such as Mango, Litchi, Banana, Rambai, Date and Guava were purchased from four different markets in Dhaka city. Such as, Cantonment market (Nabinagar), Savar kacha Bazar, kathalbagan Bazar and from a branded grocery store named Sapno.

2.2. Sources of Microorganisms

Each selected fruit sample in intact condition was placed in separated, sterile aluminum foil paper covered, beakers containing 500 ml of distilled water, formalin, acetic acid and salt [isotonic (0.9% NaCl), hypertonic (2.5% NaCl) and hypotonic (0.5% NaCl)] solution respectively and kept for one hour. After the time interval the fruits were removed and the residual distilled water and solutions were used as the sources of microorganisms.

2.3. Culture of Microorganisms

From each beaker 1 mL sample was taken by using a sterile pipette in a sterile test tube containing 9 mL sterile distilled water to make 1/10 time diluted sample. This process was done up to 6 successive repeats to make more diluted samples. From the diluted samples, 1 mL sample was taken to culture the microorganisms both in nutrient agar plates using pour plate method and nutrient broth. After seeding microorganisms the nutrient broth and agar plates were incubated at 37°C for 24 hours. It was noteworthy to mention that in the case of formalin and vinegar, no growth of microorganism was observed on nutrient agar and Mueller Hinton agar plates. But numerous growths of microorganisms were observed regarding distilled water and isotonic salt solution though reduced microbial growth was observed for hypertonic and hypotonic salt solutions. The results were given in result section.

3. Results

3.1. Identification of Bacteria from Fruit Samples

Six different fruits samples were used in this study. All the fruit samples showed substantial numbers of bacterial colonies. From these samples six different bacterial species were isolated. The most frequently isolated bacterial species included gram negative *Klebsiella pneumoniae* (25%), *Escherichia coli* (21%), *Serratia marcescens* (12.5 %), *Pseudomonas aeruginosa* (17%) and Gram positive *Bacillus cereus* (16.5%), *Staphylococcus aureus* (8%).

3.2. Results of Biochemical Tests

Table 1. Results of Biochemical and Carbohydrate fermentation test of isolated organisms from mango. The table indicated the presence of *Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Bacillus cereus* on the surface of mango (Sample name: Mango)

Sl no	Oxi dase test	Cata Lase test	Coa Gul ase	MR	VP	Indole	Nitr ate	Citra te	Ure ase	TSI	Starch hydr olysis	Moti lity	La cto se	Glu cose	Sucr ose	Interpretation
1F	-	+	-	+	-	+	+	-	-	A/A	-	+	A/G	AG	A+	*Escherichia coli*
2F	-	+	-	-	-	-	+	+	+	-	-	-	AG	AG	AG	*Klebsiella pneumoniae*
3F	-	+	-	+	-	+	+	-	-	A/A	-	+	A/G	AG	A+	*Escherichia coli*
4F	+	+	-	-	-	-	+	+	-	-	-	+	-	AG	AG	*Serratia marcescens*
5F	-	+	-	-	+	-	+	+	-	K/A	+	+	-	-	A	*Bacillus cereus*

Here: K/A = alkaline slant and acidic butt, K/N =alkaline slant and neutral butt, A/A =acidic slant and acidic butt, TSI= Triple sugar iron.

Table 2. Results of biochemical and carbohydrate fermentation test of isolated organisms from litchi. The table indicated the presence of *Klebsiella pneumoniae, Serratia marcescens, Bacillus cereus, Staphylococcus aureus* on the surface of litchi (Sample name: Litchi)

Sl no	Oxidase test	Catalase test	Coagulase test	MR	VP	Indole	Nitrate	Citrate	Urease	TSI	Starch hydrolysis	Motility	Lactose	Glucose	Sucrose	Interpretation
FR1	-	+	-	+	-	+	+	-	-	A/A	-	+	AG	AG	A+	*Escherichia coli*
FR2	-	+	-	-	+	-	+	+	-	K/A	+	+	-	AG	A	*Bacillus cereus*

Table 3. Results of biochemical and carbohydrate fermentation test of isolated organisms from rambai. The table indicated the presence of *Bacillus cereus, Escherichia .coli* on the surface of rambai (Sample name: Rambai (Barmiz grapes))

Sl no	Oxidase test	Catalase test	Coagulase test	MR	VP	Indole	Nitrate	Citrate	Urease	TSI	Starch hydrolysis	Motility	Lactose	Glucose	Sucrose	Interpretation
1G	+	+	-	-	-	+	+	+	-	K/N	-	-	-	-	-	*Pseudomonas aeruginosa*
2G	-	+	-	+	-	+	+	-	-	A/A	-	+	AG	AG	A	*Escherichia coli*

Table 4. Results of biochemical and carbohydrate fermentation test of isolated organisms from guava. The table indicated the presence of *Pseudomonas aeruginosa , Escherichia .coli* on the surface of guava (Sample name: Banana (Sample name: Guava)

Sl no	Oxidase test	Catalase test	Coagulase test	MR	VP	Indole	Nitrate	Citrate	Urease	TSI	Starch hydrolysis	Motility	Lactose	Glucose	Sucrose	Interpretation
1B	-	+	-	-	-	-	+	+	+	-	-	-	AG	AG	AG	*Klebsiella pneumoniae*
2B	+	+	-	-	-	+	+	+	-	K/N	-	-	-	-	-	*Pseudomonas aeruginosa*

Table 5. Results of biochemical and carbohydrate fermentation test of isolated organisms from banana, collected from Nabinagar bazar. The table indicated the presence of *Klebsiella pneumoniae, Pseudomonas aeruginosa* on the s urface of banana, collected from Nabinagar bazar (Sample name: Banana (collected from Nabinagar))

Sl no	Oxidase test	Catalase test	Coagulase test	MR	VP	Indole	Nitrate	Citrate	Urease	TSI	Starch hydrolysis	Motility	Lactose	Glucose	Sucrose	Interpretation
3B	+	+	-	-	-	+	+	+	-	K/N	-	-	-	-	-	*Pseudomonas aeruginosa*
4B	-	+	-	-	-	-	-	+	+	K/A	+	+	-	+	*A*	*Bacillus cereus*

Table 6. Results of biochemical and carbohydrate fermentation test of isolated organisms from banana, collected from Dhaka. The table indicated the presence of *Bacillus cereus Pseudomonas aeruginosa* on the surface of banana, collected from Dhaka (Sample name: Banana (collected from Dhaka))

Sl no	Oxidase test	Catalase test	Coagulase test	MR	VP	Indole	Nitrate	Citrate	Urease	TSI	Starch hydrolysis	Motility	Lactose	Glucose	Sucrose	Interpretation
D1	-	+	-	+	-	+	+	-	-	A/A	-	+	AG	AG	A+	*Escherichia coli*
D2	-	+	-	-	-	-	+	+	+	-	-	-	AG	AG	AG	*Klebsiella pneumoniae*
D3	+	+	-	-	-	+	+	+	-	K/N	-	-	-	-	-	*Pseudomonas aeruginosa*
D4	-	+	-	-	-	-	+	+	-	K/A	+	+	-	AG	A	*Bacillus cereus*
D5	-	+	+	-	+	-	+	+	-	A/A	-	-	AG	AG	AG	*Staphylococcus aureus*

Table 7. Results of biochemical and carbohydrate fermentation test of isolated organisms from dates, collected from Nabinagar Bazar (Sample Name: Dates (collected from Nabinagar Bazar)

Sl no	Oxidase test	Catalase test	Coagulase test	MR	VP	Indole	Nitrate	Citrate	Urease	TSI	Starch hydrolysis	Motility	Lactose	Glucose	Sucrose	Interpretation
Ds	+	+	-	-	-	+	+	+	-	K/N	-	-	-	-	-	*Pseudomonas aeruginosa*

The table indicated the presence of Escherichia .coli, Klebsiella pneumoniae Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus on the surface of dates, collected from Nabinagar bazar.

Table 8. Results of biochemical and carbohydrate fermentation test of isolated organisms from dates collected from super shop, Shapno (Sample name: Dates (collected from a super shop Shapno, Dhaka))

Sl no	Oxidase test	Catalase test	Coagulase test	MR	VP	Indole	Nitrate	Citrate	Urease	TSI	Starch hydrolysis	Motility	Lactose	Glucose	Sucrose	Interpretation
Ds	+	+	-	-	-	+	+	+	-	K/N	-	-	-	-	-	*Pseudomonas aeruginosa*

The table indicated the presence of *Pseudomonas aeruginosa* on the surface of dates, collected from Shopno , a supershop, Dhaka.

3.3. The Effects of Vinegar and Salt on Microbial Growth

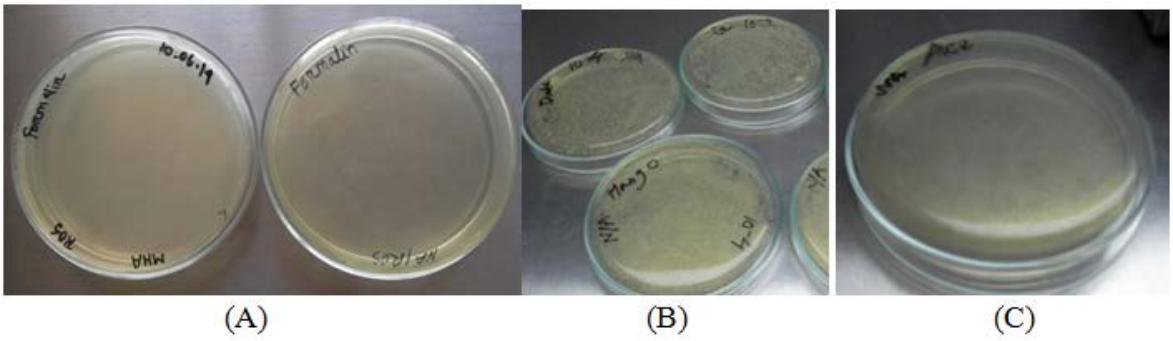

Figure 1. (A). No growth was found after washing with formalin(40% formaldehyde), (B). Numerous growth was found after washing with distilled water, (C). No growth was found after washing with vinegar (10% acetic acid)

A numerous growth of microorganisms was found after washing with isotonic solution of NaCl (0.9%) but the growth of microorganisms were decreased after washing with hypotonic (0.5%) and hypertonic (2.5%) solution of NaCl.

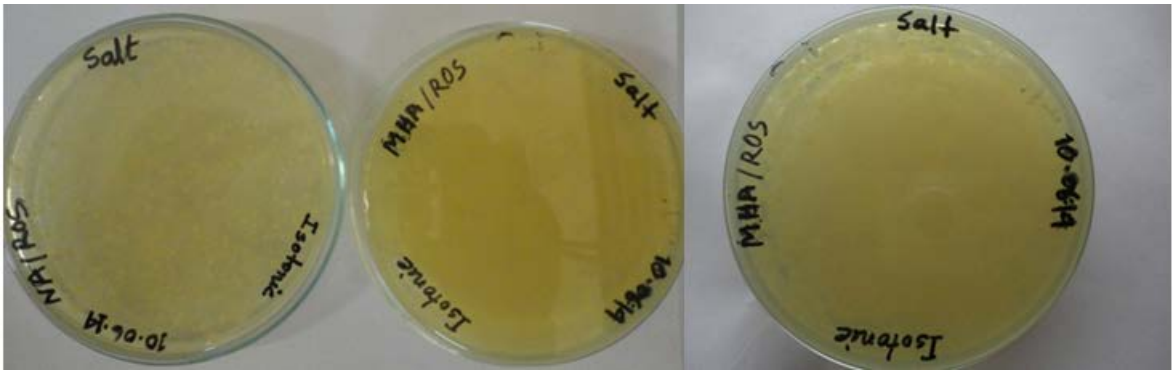

Figure 2. A numerous growth was found after washing with isotonic solution

Figure 3. Growth of microorganism was decreased after washing with hypertonic and hypotonic solution

4. Discussion

Our results demonstrated high bacterial diversity in the six verities of fruits. Among the bacterial species gram negative bacteria like *Escherichia coli, Klebsiella pneumoniae, Serratia marcescens, Pseudomonas aeruginosa and Gram positive bacteria Bacillus cereus, Staphylococcus aureus* etc. were dominating. Enterotoxigenic *E. coli* is a cause of traveller's diarrhoea. Contaminated raw fruits are thought to be a common cause of traveller's diarrhea [13]. When improperly composted cow manure has been applied as a fertilizer the potential for contamination enhanced. Workers on farms and in packing houses were also source of *E. coli*. *Klebsiella pneumoniae* (*K. pneumoniae)* is usually found in the normal flora of skin, mouth, and intestines and one of the most important bacteria which is responsible for pneumonia (the destructive lung inflammation disease) [2]. *Klebsiella* is an opportunistic pathogens that primarily attacks immunocompromised individuals and hospitalized patients [15]. Consumption of contaminated fresh fruits with *K. pneumoniae* can represent a potential risk to consumer's health. Like an opportunistic human pathogen that can cause disease in animals, including humans [4]. *S. marcescens* may cause extrinsic staining of the teeth also [6]. Foods like raw fruits are generally linked to illness implicating *B. cereus*. Illness associated with eating contaminated fruits tends to be restricted to self-limiting diarrhoea (enterotoxin) or vomiting (emetic toxin). However, emetic toxin-producing strains have produced liver failure and death by the foodborne route [16]. *Staphylococcus aureus* is known to be carried in the nasal passages of healthy food handlers and has been detected on fruits [1]. It is difficult to unequivocally determine the specific factors responsible for driving the divergence between the bacterial communities on different fruit types, but several factors contributed to the patterns observed. Differences in handling, transport, and storage could also play a role in structuring the microbial communities. Furthermore, differences in storage temperatures among

fruit items due to refrigeration could influence the relative abundance of cold-tolerant bacteria. Additional research needs to be conducted to disentangle the contribution of these factors in structuring produce-associated bacterial communities [9]. A consequence of inappropriate manipulation and storage conditions, both pathogenic and deteriorative microorganisms may contaminate fruits and increase the risk of microbial diseases and spoilage. One effective way of decreasing microorganisms from fruit surface showed here, that was washing fruits with vinegar (acetic acid) or hypotonic or hypotonic solution of salt (NaCl).Formaldehyde not only disinfected the tissue but replaced the tissue cell moisture with a rigid gel. Additionally, the “new” cell structure resisted further bacterial attacks [17]. Vinegar (acetic acid) used here as preservative. Vinegar penetrated the cell membrane lipid bi layer easily. Once inside the cell, the acid was forced to dissociate into charged anions and protons because the cell interior had a higher pH than the exterior. Protons generated from intracellular dissociation caused a progressive decline in intracellular pH, which, in turn, inhibited glycolysis , affect cell signaling, and inhibited active transport [21]. Vinegar also interfered with membrane permeability. Thus, acetic acid interfered with energy metabolism by alteration of the structure of the cytoplasmic membrane due to an interaction with membrane proteins [20]. Our study supported the hypothesis that vinegar was the better preservative than formalin and salt.

5. Conclusion

Our study demonstrated that several bacteria found on fruits surfaces are capable to cause diseases to human. Differences in surface characteristics, type, physiological state of fruits, type of farming practice, possibility of contamination during harvesting, handling and processing and environmental conditions influence the presence of various microorganisms on fruit surfaces. From the study it might be concluded that washing fruits in potable water removed a portion of microbial cells and vigorous washing with vinegar can be an effective treatment. Washing fruits with hypotonic and hypertonic solution of salt (NaCl) might be an effective way to reduce bacterial load. Water containing higher concentration of salt generally reduced the bacterial populations many folds. Heavily contaminated fruits should be subjected to a double wash treatment. Success in removing soil or faecal matter, and the contaminants there in, was more likely to be achieved by first washing in potable water and then washing or rinsing in water containing vinegar or salt. During this study we also observed that fruits dipped in acetic acid for 30 minutes reduced bacterial populations and increased the shelf life of fruits (Figures were not shown). So it may be concluded that vinegar may be a better preservative instead of formalin in respect of harmful effects of formalin.

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